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EXAMINER	
FORD, ALLISON M	

ART UNIT	PAPER NUMBER
1651	

DATE MAILED: 12/13/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/595,195	NIGAM ET AL.	
	Examiner	Art Unit	
	Allison M Ford	1651	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *21 September 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-12* is/are pending in the application.

4a) Of the above claim(s) *2-4,8,9 and 11* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,5-7,10 and 12* is/are rejected.

7)☒ Claim(s) *1 and 5* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

Applicant's election without traverse of Group I, claims 1, 5-7, 10 & 12 in the reply filed on 9/21/04 is acknowledged. Claims 1-12 are pending in the current application, of which claims 2-4 & 8-9 have been withdrawn from consideration.

Drawings

The drawing of Figure 2 is objected to as failing to comply with 37 CFR 1.84(p)(5) because it includes the following reference character(s) not mentioned in the description: 1-5. A corrected drawing sheet in compliance with 37 CFR 1.121(d), or amendment to the specification to add the reference character(s) in the description in compliance with 37 CFR 1.121(b) are required in reply to the Office action to avoid abandonment of the application. Any amended replacement drawing sheet should include all of the figures appearing on the immediate prior version of the sheet, even if only one figure is being amended. The replacement sheet(s) should be labeled "Replacement Sheet" in the page header (as per 37 CFR 1.84(c)) so as not to obstruct any portion of the drawing figures. If the changes are not accepted by the examiner, the applicant will be notified and informed of any required corrective action in the next Office action. The objection to the drawings will not be held in abeyance.

Figures 2-8 should be designated by a legend such as --Prior Art-- because it appears only that which is old is illustrated. See MPEP § 608.02(g). Corrected drawings in compliance with 37 CFR 1.121(d) are required in reply to the Office action to avoid abandonment of the application. The replacement sheet(s) should be labeled "Replacement Sheet" in the page header (as per 37 CFR 1.121(d)) so as not to obstruct any portion of the drawing figures. If the changes are not accepted by the examiner, the applicant will be notified and informed of any required corrective action in the next Office action. The objection to the drawings will not be held in abeyance.

Specification

Throughout the specification applicant uses numbers in parenthesis, as if to refer to numbered references; however, there does not appear to be a numbered list of references provided in the specification. The listing of references on the IDS submitted 3/15/2004 and 9/15/2003 are proper listings, therefore it is not required the references also be listed in the specification; rather, it would be more proper to delete these reference numbers from the specification, or if specific reference is wished to be had following the appropriate information, entering the entire citation in place of the reference number would also be proper.

The use of the trademark Matrigel has been noted in this application. It should be capitalized wherever it appears and be accompanied by the generic terminology.

Although the use of trademarks is permissible in patent applications, the proprietary nature of the marks should be respected and every effort made to prevent their use in any manner which might adversely affect their validity as trademarks.

The title of the invention is not descriptive. A new title is required that is clearly indicative of the invention to which the claims are directed. The following title is suggested: METHOD OF FORMING VASCULARIZED KIDNEY TISSUE.

Finally, in order to facilitate prosecution the examiner has assigned unofficial letterings to the steps in claim 1. This will allow the examiner to more clearly make reference to a specific step of the method; no changes have been made to the claim language. Claim 1 will be referred to as follows:

1. A method for constructing a functional mammalian organ or a fragment thereof in vitro, comprising:

(a) culturing and propagating embryonic epithelial-derived explants, tissue or cells comprising:

(i) isolating the tissues or cells and growing them in culture;

(ii) permitting the culture to form multiple branches;

(iii) dissecting out individual branch tips;

(iv) reculturing the individual branch tips in the presence of serum, a growth factor mix, conditioned medium and nutrient rich medium for several generations to generate branch tip buds;

(b) simultaneously culturing and propagating isolated embryonic or fetal metanephric mesenchyme, comprising:

(i) dissecting out fetal mesenchyme at the time of induction;

(ii) culturing mesenchymal tissue in the presence of serum, a growth factor mix, conditioned medium and nutrient-rich medium;

(iii) partitioning the mesenchyme into multiple pieces and growing each piece separately;

(iv) inducing vasculogenesis by subjecting grown mesenchyme to substrate deprivation or addition or soluble factors;

(c) recombining each vascularized mesenchyme with each cultured bud in a matrix in which in vitro angiogenesis has begun; and

(d) growing under conditions to ensure continued vasculogenesis.

Claim Objections

Applicant's claim 1 is objected to because of a minor informality due to a grammatical error, in step (b)(iv) of the amended claim it appears it should read, "inducing vasculogenesis by subjecting grown mesenchyme to substrate deprivation or addition of soluble factors."

Applicant's claim 5 is missing a period at the end of the claim following "thereof".

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claim 1 is rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of constructing a vascularized kidney tissue comprising tubularized metanephric epithelium and metanephric mesenchyme, it does not reasonably provide enablement for a method for constructing a functional mammalian organ. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

Applicant's claim 1 is directed to a method which will create a vascularized kidney tissue construct, comprising metanephric mesenchyme combined with cultured branch tip buds from ureteric bud cells, wherein the UB structures were shown to elongate into the mesenchymal structure (See Specification Pg 20 & 26). The functional mammalian kidney is a complex and highly specialized organ capable of filtrative, reabsorptive, homeostatic and endocrine functions; for function within the body it must also be integrated into the various systems of the body, including the circulatory system, for providing oxygen and nutrients, and the genitourinary system. Kidney organogenesis involves many complex steps, beginning with differentiated nephrogenesis, followed by tubulogenesis of the newly-formed-epithelium, which applicant has demonstrated with the vascularization of the UB structures incorporated in the mesenchyme; however, complete development further requires lumen formation, the appearance of the A chain of laminin in the basal regions of the mesenchymal cell aggregates, and more series of sequential growths and differentiations (See Humes, US Patent 6,060,270 col. 1, ln 45-58). While there is evidence in the art of formation of partial organs, such as a renal tubule tissue system of Humes, there is no evidence of formation of a complete, functional kidney formed in vitro. Therefore, while applicant provides a method to develop vascularized renal tissue, their examples do not show evidence of a functional organ, capable of performing as a functional organ. Thus, due to the absence of

working examples of functional organs, lack of guidance and teachings from the current application and from prior art, the extreme complexity of the invention, and the breadth of the claims, drawn to all functional mammalian organs, applicant is limited to only a method of forming a vascularized kidney tissue comprising tubularized metanephric epithelium and metanephric mesenchyme, and not a functional mammalian organ.

Furthermore, claim 10 is rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Applicant's claim 10 requires the functional mammalian organ, created by the method of claim 1, to be implanted into a recipient without prior induction of vascularization. Applicant is not enabled for producing a functional mammalian organ, for the reasons stated above. However, beyond producing a functional organ, in order to transplant it into a recipient one must provide evidence that they have overcome the numerous issues of incorporating the organ into the surrounding systems of the body, and more difficult still, preventing rejection by the body. Preventing rejection is one of the primary concerns with whole organ transplants, applicant has provided no evidence or any teachings or guidance on how this monumental obstacle is overcome, therefore, in the lack of any experimental evidence, there is no reasonable expectation of success of this transplant without detailed information on compatibility or immunsuppression therapy. Organ transplant is an extremely unpredictable art, examples in the prior art have had limited success getting organs to be accepted and remain viable after significant periods of time. Therefore, due to the complexity of the nature of the claim, the absence of working examples and lack of guidance, and the extreme unpredictability of the art, and lack of enablement for forming the functional organ, there is not sufficient evidence to provide enablement for successful implantation of the tissue.

Claim 6 is rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Applicant describes a method for inducing vasculogenesis of epithelial derived cells in metanephric mesenchymal tissue by the steps enumerated above. Cells are to be cultured in a conditioned medium, which is defined in the specification to be the culture medium obtained by washing a confluent monolayer of BSN cells with serum-free medium, culturing the BSN cells in serum free medium for 2-4 days, and then harvesting and concentrating the medium (See Specification Pg 18-19). Applicant teaches that the BSN culture medium (BSN-CM) has an unidentified growth promoting constituent and/or inducer of differentiation. However, because this factor remains unidentified applicant is relying on the inherent property of the factors found in the medium; they have not provided sufficient written description and characterization of the growth promoting constituent and/or inducer of differentiation present in the BSN-CM to adequately identify these constituents (See Specification Pg 9). Applicant has not provided the necessary disclosure of relevant, identifying characteristics, such as structure or other physical or chemical properties, or functional characteristics, beyond disclosure of the generic action inherent to the growth medium, sufficient to show the applicant was in possession of the claimed matter. *See Eli Lilly,* 119F. 3d. at 1568, 43 USPQ2d at 1406. See MPEP § 2163.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 1 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In applicant's amendment of claim 1, without the letterings added for examination purposes by the examiner, applicant claims "simultaneously culturing and propagating isolated embryonic or fetal metanephric mesenchyme;" as written it is confusing with which previous step this culturing is simultaneously being carried out. Additionally, as written, it was extremely confusing whether or not the steps of recombining each vascularized mesenchyme with each cultured bud and growing under conditions to ensure continued vascularization were separate steps or if they were steps of culturing the mesenchymal tissue. It would be remedial to adopt the letter/enumerating system suggested by the examiner, or one similar, as applicant sees fit, or otherwise incorporating into the claim exactly which step this culturing is simultaneous to.

Applicant's claim 1(a)(ii) reads, "permitting the culture to form multiple branches;" it is unclear what is meant by the culture forming branches. Most cells or tissues will grow to a confluent monolayer, it is not clear if these cultures are to form dendrite-like projections, or what induces them to form what applicant calls 'branches.'

Applicant's claim 1 (a)(iv) requires the individual branch tips to be recultured in the presence of serum, a growth factor mix, conditioned medium and nutrient rich medium for several generations. From the specification (Pg 18-19) conditioned medium is being understood to be concentrated culture medium obtained by washing a confluent monolayer of BSN cells with serum-free medium, culturing the BSN cells in serum free medium for 2-4 days, and then harvesting and concentrating the medium. It is not clear, however, what constitutes nutrient rich medium. It would be assumed in the art that medium consisting of conditioned medium, serum, and growth factors *is* a nutrient rich medium; however, from the language of the claim it appears the nutrient rich medium is another ingredient that was added to the cell culture medium. It is not clear if the nutrient rich medium has additional factors or components, and if so, it is not clear what these additional nutrients are.

Applicant's claim 1(b)(i) requires fetal mesenchyme to be dissected out at the time of induction. It is not clear what part of the mesenchyme is being dissected out; mesenchyme describes many types of tissue, including organs, blood, muscle, connective tissue, and more, if this fetal mesenchyme the fetal metanephric mesenchyme described in step (b) it should be referred to as fetal metanephric mesenchyme throughout. Furthermore, it is not clear why applicant chose to particularly point out that the dissection of fetal mesenchyme occurred at the time of induction. It seemed this step was to occur simultaneously to step (a)(i); however because it was not specifically stated in step (a)(i) that the isolation occurred at the time of induction, it appears as if the isolation of step (a)(i) did not occur at the time of induction, and the two steps were not simultaneous.

Applicant's claim 1(b)(ii) requires mesenchymal tissue to be cultured in the presence of serum, a growth factor mix, conditioned medium and nutrient rich medium. Again, it is not clear if this mesenchymal tissue is the same as the fetal metanephric mesenchyme in step (b) or the fetal mesenchyme dissected out in step (b)(i), the same terminology should be used throughout. Additionally, the same question is raised again about the content of the nutrient rich medium, as in step (a)(iv). While the composition of conditioned medium is explained in the text, nutrient rich medium is not. Therefore, it would appear that medium consisting of conditioned medium, serum, and growth factors *is* a nutrient rich medium; however, from the language of the claim it appears the nutrient rich medium is another ingredient that was added to the cell culture medium. It is not clear if the nutrient rich medium has additional factors or components, and if so, it is not clear what these additional nutrients are.

Applicant's claim 1(b)(iii) requires the partitioning of the mesenchyme into multiple pieces, and growing of each piece separately. Again, it is not clear if this mesenchyme is the same as the fetal metanephric mesenchyme in step (b), the fetal mesenchyme dissected out in step (b)(i) or the mesenchyme tissue in step (b)(ii), the terminology should remain consistent throughout. Additionally, it

appears it would be more accurate if the term "culturing" replaced the term "growing" to remain consistent.

Applicant's claim 1(b)(iv) requires subjecting grown mesenchyme to substrate deprivation or addition of soluble factors. Again, it is not clear if this mesenchyme is the same as the fetal metanephric mesenchyme in step (b), the fetal mesenchyme dissected out in step (b)(i), the mesenchyme tissue in step (b)(ii), or the mesenchyme from step (b)(iii), the terminology should remain consistent throughout. It is further unclear what constitutes grown mesenchyme, the cells were always fully grown, it appears the applicant intends to refer to a certain time period which must pass, or a certain confluency level, or a size of a forming tissue which must be reached, however, as is, it is not clear what 'grown mesenchyme' is. Still further, it was not clear that the fetal metanephric mesenchyme was initially cultured on a substrate, so it is not clear how vasculogenesis is induced by substrate deprivation. Finally, it is not clear what constitutes the soluble factors which are added to induce vasculogenesis.

Applicant's claim 1(c) requires recombining each vascularized mesenchyme with each cultured bud in a matrix. It is unclear if the cultured buds are the same as the branch tip buds in step (a)(iv), the same terminology must be used throughout. Additionally, it is not clear if the vascularized mesenchyme is the same as the fetal metanephric mesenchyme in step (b), the fetal mesenchyme dissected out in step (b)(i), the mesenchyme tissue in step (b)(ii), or the grown mesenchyme of step (b)(iv), the same terminology must be used throughout. Furthermore, it is not clear how the buds and mesenchyme are *re*combined, as it was not clear that they were ever together, there is no indication the embryonic epithelial-derived cells and the fetal metanephric mesenchyme even came from the same source. It is also not clear how the bud and mesenchyme are combined, if they are placed next to one another, if they are infused in some way, if a physical connection is made, etc, this combining is not clear. The specification only teaches the Ureteric Buds were "recombined with freshly isolated E-13 rat metanephric

mesenchyme" (See Specification Pg 20), it does not provide any information on how the recombination occurs.

Finally, applicant's claim 1(d) requires growing under conditions to ensure continued vasculogenesis. Though it can be assumed that it is the bud-mesenchyme combination that is to be grown, it needs to be clearly pointed out and distinctly claim that it is the tissue combination that is being grown. Furthermore, it is not clear what comprises the conditions that ensure continued vasculogenesis. The specification teach the combined Ureteric buds and metanephric mesenchyme are cocultured on a transfilter for 5 days in DMEM/F12, plus 10% FCS (See Specification, Pg 20); though it is not clear if vasculogenesis has occurred at this point, and if so, if these conditions ensure continued vasculogenesis, it is being assumed for the purposes of examination that continued coculture on a transfilter in DMEM/F12 plus 10% FCS are the conditions for vasculogenesis.

Claim 1 is further rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. The omitted steps are: the culture of the epithelial-derived explants, tissue or cells must be cultured with a polycarbonate membrane insert and an extracellular matrix (ECM) gel must be applied on top of the insert. Applicant teaches use of the insert and ECM gel during the culturing of Ureteric Buds in example 1 (See Specification, Pg 18). The insert and ECM gel appear to be critical for the proper branching of the Ureteric Buds, as the UB grow on the interface of air and culture medium. The inclusion of an ECM gel and polycarbonate insert should be claimed in step (a).

Claims 5-7, 10 and 12 have the limitation of claim 1, and therefore are rejected on the same basis.

Applicant's claim 5 requires the growth factor mix to comprise a glial cell line derived neurotrophic factor or functional equivalent thereof. It is not clear what other growth factors are included to create a growth factor *mix*, as applicant only requires the one glial cell line derived neurotrophic factor. In the specification applicant describes in the discussion that a mix of EGF, HGF, IFG, FGF-2 and GDNF

was required for dichotomous branching morphogenesis, however this mix was not taught in the examples. It appears the mix of EGF, HGF, IFG, FGF-2 and GDNF is critical for the success of the invention, therefore applicant must clearly point out and claim the complete growth factor mix. Furthermore, it is not clear what constitutes a functional equivalent of this glial cell line derived neurotrophic factor, as the neurotrophic factor is not characterized in a way sufficient to allow one of ordinary skill in the art to confidently select an alternative growth factor with equivalent functionality.

Applicant's claim 6 requires the conditioned medium to comprise a growth promoting constituent or inducer of differentiation or morphogenesis. The specification defines conditioned medium as being concentrated culture medium obtained by washing a confluent monolayer of BSN cells with serum-free medium, culturing the BSN cells in serum free medium for 2-4 days, and then harvesting and concentrating the medium (See Specification Pg 18-19). This definition does not provide evidence of a growth promoting constituent or inducer of differentiation; therefore it is not clear what these growth promoting constituents or inducers of differentiation consist of. Therefore the claim fails to particularly point out and claim the invention.

Claim 7 contains the trademark/trade name Matrigen, the correct trademark name is "Matrigel." Furthermore, where a trademark or trade name is used in a claim as a limitation to identify or describe a particular material or product, the claim does not comply with the requirements of 35 U.S.C. 112, second paragraph. See *Ex parte Simpson*, 218 USPQ 1020 (Bd. App. 1982). The claim scope is uncertain since the trademark or trade name cannot be used properly to identify any particular material or product. A trademark or trade name is used to identify a source of goods, and not the goods themselves. Thus, a trademark or trade name does not identify or describe the goods associated with the trademark or trade name. In the present case, the trademark/trade name is used to identify/describe a type of basement membrane extract and, accordingly, the identification/description is indefinite.

Applicant's claim 10 requires the tissues to be implanted into a recipient without prior induction of vasculogenesis. It is not clear which tissues this claim is referring to, the freshly isolated epithelial-derived tissue (claim 1, step (a)(i)), the branched tip buds (claim 1, step (a)(iv)), the isolated embryonic or fetal metanephric mesenchyme (claim 1, step (b)(i)), the grown mesenchyme undergoing vasculogenesis due to substrate deprivation or addition of soluble factors (claim 1, step (b)(iv)), or the recombined vascularized mesenchyme and buds (claim 1, step (c)). Furthermore, it is not clear when this step occurs, as claim 1 requires vasculogenesis to have at step (b)(iv), which occurs even before the recombination of embryonic epithelial-derived explants, tissue or cell and the fetal metanephric mesenchyme in step (c), thus the recombined tissue cannot be implanted. Therefore claim 10 does not include all the limitations of claim 1, on which it is dependent, because steps (c) and (d) cannot be completed before the vascularization of the combined tissue.

Applicant's claim 12 requires the tissue to be mammalian kidney tissue. Again, it is not clear which tissues this claim is referring to, the freshly isolated epithelial-derived tissue (claim 1, step (a)(i)), the branched tip buds (claim 1, step (a)(iv)), the isolated embryonic or fetal metanephric mesenchyme (claim 1, step (b)(i)), the grown mesenchyme undergoing vasculogenesis due to substrate deprivation or addition of soluble factors (claim 1, step (b)(iv)), or the recombined vascularized mesenchyme and buds (claim 1, step (c)).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 5-7 & 12 are rejected under 35 U.S.C. 103(a) as being unpatentable over Sakurai et al (*PNAS*, 1997), in view of Sariola et al (US Patent 5,882,923).

Sakurai et al teach a method of inducing Ureteric Bud (UB) cell tubulogenesis (induction of epithelial tubules in the mesenchyme), comprising obtaining UB cells from a day 11.5 mouse embryo and culturing the cells in a three-dimensional growth factor-depleted ECM gel of Matrigel and type I collagen (See Pg. 6280, col. 1); and then placing day 13 embryonic kidneys on top of the ECM gels. The embryonic kidneys produce soluble factors that induce UB cell morphogenesis, characterized by UB cell processes and multicellular cord formation (which applicant calls construction of a functional mammalian organ) (See pg 6281, col. 2).

In another embodiment Sakurai et al teach adding BSN culture media (BSN-CM)to the UB cells in the three dimensional ECM gel culture and observed tubulogenesis results comparable to those induced by the embryonic kidney (See Pg 6281, col. 2). The BSN-CM contained the soluble factors from the mesenchymal cell line derived from the embryonic kidney (See Pg 6282, col. 2). The BSN-CM was obtained in the same fashion as the current application, a confluent cell monolayer of BSN cells was washed twice with serum-free DMEM/F12 medium, followed by application of serum-free DMEM/F12 and incubated for 2-4 days; the collected BSN-CM was centrifuged at low speeds to remove cell debris (See Sakurai et al Pg 6280, col. 1 & Specification Pg 18-19).

Therefore Sakurai et al teach culturing and propagating UBs obtained from embryonic mice, which are embryonic epithelial-derived cells from mammalian kidneys (Claim 12); the buds are cultured directly in a three dimensional matrix comprised of type I collagen and Matrigel (Claim 7); the Ureteric Buds in the matrix are then combined with embryonic kidneys, which is isolated embryonic mesenchyme which has already begun vasculogenesis (Claim 1(c)); and the combined system is maintained in conditions that ensured continued vasculogenesis (Claim 1(d)).

Though the steps of claim 1 differ slightly in steps (a) and (b), the same general effect is created. Sakurai et al isolated and maintained Ureteric Buds from embryonic mouse kidneys in suitable culture conditions. Though they do not clearly state they isolated individual branch tip buds by permitting the culture to form multiple branch tips, dissecting out individual branch tips, and reculturing the individual branch tips to generate branch tip buds, they do teach using Ureteric Bud cells and it appears they would have been isolated in the same manner. However, even if Sakurai et al did not isolate the UB cells in the same manner as delineated in claim 1(a), it would have been obvious to one of ordinary skill in the art at the time the invention was made to isolate individual branch tips by permitting a culture of UB cells to form multiple branch tips, dissecting out individual branch tips, and reculturing the individual branch tips to generate branch tip buds. One of ordinary skill in the art would have been motivated to isolate the branch tip buds in this manner because it ensures a high number of UBs, because they are continually split and recultured, which allows them to multiply. One would have expected success because suitable culture medium and conditions were used, and splitting cells for the purpose of multiplication is well known in the cell culture art (Claim 1(a)).

Though Sakurai et al chose to use whole, isolated, embryonic kidneys instead of dissecting out fetal metanephric mesenchyme, culturing the mesenchymal tissue, partitioning it into multiple pieces, growing each piece out, and subjecting the pieces to substrate deprivation or addition of soluble factors to induce vasculogenesis to produce vascularized metanephric mesenchyme tissue, it would have been obvious to one of ordinary skill in the art at the time the invention was made to produce vascularized mesenchyme by any appropriate method. Both the method of the current application, and Sakurai et al's method of using a whole embryonic kidney, produce a vascularized metanephric mesenchymal tissue that secrete the soluble factors that, in turn, induce tubulogenesis in the UB cells. One would have been motivated to produce vascularized metanephric mesenchymal tissue, obtained by dissecting out fetal metanephric mesenchyme tissue, culturing the tissue, partitioning it into multiple pieces, growing each

piece out, and subjecting the pieces to substrate deprivation or addition of soluble factors to induce vasculogenesis, instead of whole embryonic kidneys if the kidney was already dissected to obtain the UB cells. In order to reduce the number of experimental animals required, the same kidneys could be used to harvest the UB cells and to supply the metanephric mesenchyme; however after harvesting the UB cells significant disruption would be caused to the metanephric mesenchyme and the mesenchymal tissue would have to be recultured and expanded to provide the same effect as an intact kidney. One would expect success using recultured, vascularized metanephric mesenchymal tissue or an intact kidney because both would supply the necessary soluble factors that induce UB cell process and multicellular cord formation. These soluble factors are produced in the mesenchyme, as evidenced by the successful induction of tubulogenesis when only BSN-CM was added to the UB cells in matrix, the BSN-CM only contained soluble factors produced by the mesenchymal BSN cells (See Pg. 6381, col. 2) (Claim 1(b)).

Additionally, because of the discovery that BSN-CM contain the soluble factors necessary for induction of tubulogenesis (See Pg 6282 & Fig. 5), it would have been obvious to one of ordinary skill in the art at the time the invention was made to culture the UB cells and mesenchymal tissues in BSN-CM (Claim 1(a)(iv) and 1(b)(ii)). One of ordinary skill in the art would have been motivated to use BSN-CM as the culture medium in order to promote vascularization and tubulogenesis in the individual cell cultures and would have expected success because BSN-CM inherently contains what applicant calls growth promoting constituents and inducers of differentiation or morphogenesis, as evidenced by the morphogenesis induced by the addition of BSN-CM to the matrix containing UB cells (See Pg. 6282, col. 2) (Claim 6).

Similarly, the addition of a growth factor mix, including glial cell derived neurotrophic factor (GDNF), also initially induced UB cell morphogenesis, but its effects dwindled after prolonged time periods (See Pg 6284, col. 2 and Fig. 5). Additionally, Sariola et al teach that GDNF promotes ureteric morphogenesis by priming the Wolffian ducts for bud initiation, where the epithelial tubules will

eventually perforate the mesenchyme, perhaps by increasing cell adhesion in the mesenchyme (See col. 4, ln 49-56). Sariola et al also teach that other various growth factors, such as TBF-B1 and HGF play a role in bud elongation and propagation (See col. 4, ln 49-56). Therefore because the growth factor mix did show some positive effect on the tubulogenesis and vascularization of the UB cells in Sakurai et al's experiments, and Sariola et al teach that GDNF, in particular, is critical for ureteric morphogenesis, and other various growth factors play a role in bud elongation, it would have been obvious to one of ordinary skill in the art at the time the invention was made to add growth factor mix containing GDNF to the culture medium used for the UB cells and mesenchymal tissue (Claim 1(a)(iv) and 1(b)(ii)). One of ordinary skill in the art would have been motivated to add growth factor mix containing GDNF because the growth factor mix did show a positive effect on tubulogenesis when added to UB cells in the ECM matrix (See Sakurai et al Pg 6282, col. 2 & Fig. 5) and because GDNF is critical for early ureteric bud development (See Sariola et al, col. 7, ln 33-46); therefore one would have expected success combining the growth factor mix and contact with mesenchymal tissue and BSN-CM, which both provide the soluble factors that induce tubulogenesis and vascularization (Claim 5).

Therefore the invention as a whole would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Allison M Ford whose telephone number is 571-272-2936. The examiner can normally be reached on M-F 7:30-5.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael Wityshyn can be reached on 571-272-0926. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

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PRIMARY EXAMINER